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(71) Applicant: Ajinomoto Co., Inc.
Tokyo 104 (JP)

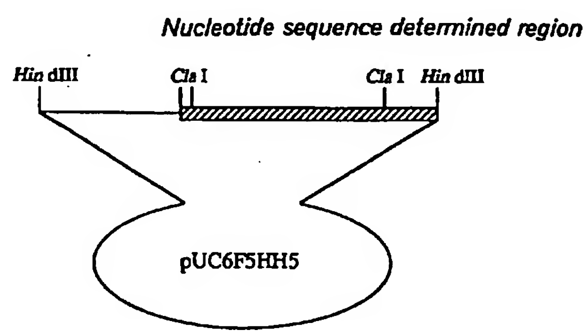
(72) Inventors:
- KIKUCHI, Yoshimi
Ajinomoto Co., Inc.
Kawasaki-ku Kawasaki-shi Kanagawa 210 (JP)
- SUZUKI, Tomoko
Ajinomoto Co., Inc.
Kawasaki-ku Kawasaki-shi Kanagawa 210 (JP)
- KOJIMA, Hiroyuki
Ajinomoto Co., Inc.
Kawasaki-ku Kawasaki-shi Kanagawa 210 (JP)

(74) Representative: Strehl Schübel-Hopf Groening & Partner
Maximilianstrasse 54
80538 München (DE)

(54) NOVEL LYSINE DECARBOXYLASE GENE AND PROCESS FOR PRODUCING L-LYSINE

(57) L-lysine is produced efficiently by cultivating, in a liquid medium, a microorganism belonging to the genus Escherichia with decreased or disappeared lysine decarboxylase activity relevant to decomposition of L-lysine, for example, a bacterium belonging to the genus Escherichia with restrained expression of a novel gene coding for lysine decarboxylase and/or a known gene cadA to allow L-lysine to be produced and accumulated in a culture liquid, and collecting it.

FIG. 1



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Description

Technical Field

The present invention relates to a novel lysine decarboxylase gene of Escherichia coli relevant to decomposition of L-lysine, a microorganism belonging to the genus Escherichia with restrained expression of the gene and/or another lysine decarboxylase gene known as cadA gene, and a method of producing L-lysine by using the microorganism. Recently, the demand of L-lysine as a feed additive actively increases.

Background Art

Lysine decarboxylase, which catalyzes a reaction to produce cadaverine by decarboxylation of L-lysine, is known as an L-lysine-decomposing enzyme of Escherichia coli. A nucleotide sequence of its gene called cadA, and an amino acid sequence encoded by the gene have been already reported (Meng, S. and Bennett, G. N., J. Bacteriol., 174, 2659 (1992)). There are two reports for lysine decarboxylase encoded by a gene other than cadA of Escherichia coli, which describe that faint activity was detected in a mutant strain of Escherichia coli (Goldemberg, S. H., J. Bacteriol., 141, 1428 (1980); Wertheimer, S. J. and Leifer, Z., Biochem. Biophys. Res. Commun., 114, 882 (1983)). However, it was reported for this activity by Goldemberg, S. H. that the enzyme activity decreased in a degree of about 30 % after a heat treatment at 60 °C for 4 minutes, while it was reported by Wertheimer, S. J. et al that no such phenomenon was observed. Accordingly, the presence of the second lysine decarboxylase is indefinite.

On the other hand, L-lysine is produced by known methods for using Escherichia coli, including a method comprising cultivating a mutant strain resistant to lysine analog or a recombinant strain harboring a vector with incorporated deoxyribonucleic acid which carries genetic information relevant to L-lysine biosynthesis (Japanese Patent Laid-open No. 56-18596). However, there is no report at all for L-lysine production by using a microorganism belonging to the genus Escherichia with restrained expression of the lysine decarboxylase gene.

Disclosure of the Invention

An object of the present invention is to obtain a novel lysine decarboxylase gene of Escherichia coli, create an L-lysine-producing microorganism belonging to the genus Escherichia with restrained expression of the gene and/or the cadA gene, and provide a method of producing L-lysine by cultivating the microorganism belonging to the genus Escherichia. When the present inventors created an Escherichia coli strain in which the cadA gene as a known lysine decarboxylase gene was destroyed, it was found that cadaverine as a decomposition product of L-lysine by lysine decarboxylase was still produced in this microbial strain Thus the present inventors assumed that a novel lysine decarboxylase gene should be present in Escherichia coli, and it might greatly affect fermentative production of L-lysine by using a microorganism belonging to the genus Escherichia. As a result of trials to achieve cloning of the gene, the present inventors succeeded in obtaining a novel lysine decarboxylase gene different from the cadA gene. It was also found that the L-lysine-decomposing activity was remarkably decreased or disappeared, and the L-lysine productivity was significantly improved by restraining expression of this gene, and restraining expression of the cadA gene in an L-lysine-producing microorganism of Escherichia coli. Thus the present invention was completed.

Namely, the present invention provides a novel gene which codes for lysine decarboxylase originating from Escherichia coli. This gene has been designated as "ldc" gene.

In another aspect, the present invention provides a microorganism belonging to the genus Escherichia having L-lysine productivity with decreased or disappeared lysine decarboxylase activity in cells.

In still another aspect, the present invention provides a method of producing L-lysine comprising the steps of cultivating, in a liquid medium, the microorganism belonging to the genus Escherichia described above to allow L-lysine to be produced and accumulated in a culture liquid, and collecting it.

The microorganism belonging to the genus Escherichia described above includes a microorganism in which lysine decarboxylase activity in cells is decreased or disappeared by restraining expression of the ldc gene and/or the cadA gene.

The present invention will be described in detail below.

(1) Preparation of DNA fragment containing novel lysine decarboxylase gene

A DNA fragment containing the novel lysine decarboxylase gene (ldc) of the present invention can be obtained as follows from an available strain of Escherichia coli, for example, K-12 strain or a derivative strain therefrom.

At first, the cadA gene, which is a gene of known lysine decarboxylase, is obtained from chromosomal DNA of W3110 strain originating from Escherichia coli K-12 by using a polymerase chain reaction method (hereinafter referred to as "PCR method"). The nucleotide sequence of the cadA gene, and the amino acid sequence encoded by it are

shown in SEQ ID NOS:5 and 6 respectively. DNA fragments having sequences similar to the cadA gene are cloned from a chromosomal DNA library of Escherichia coli W3110 in accordance with a method for using a plasmid vector or a phage vector to confirm whether or not the novel lysine decarboxylase gene is contained in the DNA fragments. The confirmation of the fact that the objective gene is contained can be performed in accordance with a Southern hybridization method by using a probe prepared by the PCR method.

A nucleotide sequence of the gene contained in the DNA fragment thus obtained is determined as follows. At first, the DNA fragment is ligated with a plasmid vector autonomously replicable in cells of Escherichia coli to prepare recombinant DNA which is introduced into competent cells of Escherichia coli. An obtained transformant is cultivated in a liquid medium, and the recombinant DNA is recovered from proliferated cells. An entire nucleotide sequence of the DNA fragment contained in the recovered recombinant DNA is determined in accordance with a dideoxy method (Sanger, F. et al., Proc. Natl. Acad. Sci., 74, 5463 (1977)). The structure of DNA is analyzed to determine existing positions of promoter, operator, SD sequence, initiation codon, termination codon, open reading frame, and so on.

The novel lysine decarboxylase gene of the present invention has a sequence from 1005-1007th ATG to 3141-3143rd GGA of the entire nucleotide sequence of the DNA fragment shown in SEQ ID NO:3 in Sequence Listing. This gene codes for lysine decarboxylase having an amino acid sequence shown in SEQ ID NO:4 in Sequence Listing. It has been found that the homology between the novel lysine decaroboxylase and the lysine decaroboxylase coded by cadA gene is 69.4 %.

The gene of the present invention may be those which code for lysine decarboxylase having the amino acid sequence shown in SEQ ID NO:4 in Sequence Listing, a nucleotide sequence of which is not limited to the nucleotide sequence described above. The lysine decarboxylase encoded by the gene of the present invention may have substitution, deletion, or insertion of one or a plurality of amino acid residues without substantial deterioration of the lysine decarboxylase activity, in the amino acid sequence described above. Genes which code for lysine decarboxylase having such deletion, insertion, or substitution can be obtained from variants, spontaneous mutant strains, or artificial mutant strains of Escherichia coli, or from microorganisms belonging to the genus Escherichia other than Escherichia coli. The mutant genes which code for lysine decarboxylase having deletion, insertion, or substitution can be also obtained by performing an in vitro mutation treatment or a site-directed mutagenesis treatment for the gene which codes for lysine decarboxylase having the amino acid sequence shown in SEQ ID NO:4. These mutation treatments can be performed in accordance with methods well-known to those skilled in the art as described below.

However, the gene, which codes for lysine decarboxylase having substitution, deletion, or insertion of one or a plurality of amino acid residues as referred to herein, includes those which originate from the "ldc gene" and can be regarded to be substantially the same as the ldc gene. It is not intended to extend the meaning to those genes having different origins. It is impossible to concretely prescribe a certain range of the "plurality". However, it will be readily understood by those skilled in the art that, for example, the cadA gene which codes for the protein different in not less than 200 amino acid residues from one having the amino acid sequence shown in SEQ ID NO:3 is different from the gene of the present invention, and the genes which code for proteins having equivalent lysine decarboxylase activity are included in the present invention even if they are different from one having the amino acid sequence shown in SEQ ID NO:3 with respect to two or three amino acid residues.

〈2〉 Creation of microorganism belonging to the genus Escherichia with restrained expression of lysine decarboxylase gene

The microorganism belonging to the genus Escherichia of the present invention is a microorganism belonging to the genus Escherichia in which the lysine decarboxylase activity in cells is decreased or disappeared. The microorganism belonging to the genus Escherichia includes Escherichia coli. The lysine decarboxylase activity in cells is decreased or disappeared, for example, by restraining expression of any one of or both of the novel lysine decarboxylase gene (ldc) and the known cadA gene described above. Alternatively, the lysine decarboxylase activity in cells can be also decreased or disappeared by decreasing or disappearing the specific activities of lysine decarboxylase enzymes encoded by these genes, by modifying the structure of the enzymes.

The means for restraining expression of the ldc gene and the known cadA gene includes, for example, a method for restraining expression of the genes at a transcription level by causing substitution, deletion, insertion, addition, or inversion of one or a plurality of nucleotides in promoter sequences of these genes, and decreasing promoter activities (M. Rosenberg and D. Court, Ann. Rev. Genetics 13 (1979) p.319, and P. Youderian, S. Bouvier and M. Susskind, Cell 30 (1982) p.843-853). Alternatively, the expression of these genes can be restrained at a translation level by causing substitution, deletion, insertion, addition, or inversion of one or a plurality of nucleotides in a region between an SD sequence and an initiation codon (J. J. Dunn, E. Buzash-Pollert and F. W. Studier, Proc. Nat. Acad. Sci. U.S.A., 75 (1978) p.2743). In addition, in order to decrease or disappear the specific activity of the lysine decarboxylase enzyme, a method is available, in which the coding region of the lysine decarboxylase gene is modified or destroyed by causing substitution, deletion, insertion, addition, or inversion of one or a plurality of nucleotides in a nucleotide sequence in the coding region.

The gene, on which nucleotide substitution, deletion, insertion, addition, or inversion is allowed to occur, may be ldc genes or cadA genes having substitution, deletion, or insertion of one or a plurality of amino acid residues which do not deteriorate the substantial activity of encoded lysine decarboxylase, in addition to the ldc gene or the cadA gene.

The method to cause nucleotide substitution, deletion insertion, addition, or inversion in the gene specifically includes a site-directed mutagenesis method (Kramer, W. and Frits, H. J., Mothods in Enzymology, 154, 350 (1987)), and a treatment method by using a chemical agent such as sodium hyposulfite and hydroxylamine (Shortle, D. and Nathans, D., Proc. Natl. Acad. Sci. U.S.A., 75, 270 (1978)).

The site-directed mutagenesis method is a method to use a synthetic oligonucleotide, which is a technique to enable introduction of optional substitution, deletion, insertion, addition, or inversion into an optional and limited nucleotide pair. In order to utilize this method, at first, a single strand is prepared by denaturing a plasmid having a cloned objective gene with a determined nucleotide sequence of DNA. Next, a synthetic oligonucleotide complementary to a portion intended to cause mutation is synthesized. However, in this procedure, the synthetic oligonucleotide is not allowed to have a completely complementary sequence, but it is designed to have optional nucleotide substitution, deletion, insertion, addition, or inversion. After that, the single strand DNA is annealed with the synthetic oligonucleotide having the optional nucleotide substitution, deletion, insertion, addition, or inversion. A complete double strand plasmid is synthesized by using T4 ligase and Klenow fragment of DNA polymerase I, which is introduced into competent cells of Escherichia coli. Some of transformants thus obtained have a plasmid containing a gene in which the optional nucleotide substitution, deletion, insertion, addition, or inversion is fixed. A recombinant PCR method (PCR Technology, Stockton press (1989)) may be mentioned as a similar method capable of introducing mutation into a gene to make modification or destruction.

The method to use the chemical agent is a method in which mutation having nucleotide substitution, deletion, insertion, addition, or inversion is randomly introduced into a DNA fragment by treating the DNA fragment containing an objective gene directly with sodium hyposulfite, hydroxylamine or the like.

Expression of the ldc gene and/or the cadA gene in cells can be restrained by substituting a normal gene on chromosome of a microorganism belonging to the genus Escherichia with the modified or destroyed gene obtained by the introduction of mutation as described above. The method for substituting the gene includes methods which utilize homologous recombination (Experiments in Molecular Genetics, Cold Spring Harbor Laboratory press (1972); Matsuyama, S. and Mizushima, S., J. Bacteriol., 162, 1196 (1985)). The homologous recombination is based on an ability generally possessed by the microorganism belonging to the genus Escherichia. When a plasmid or the like having homology to a sequence on chromosome is introduced into cells, recombination occurs at a certain frequency at a place of the sequence having the homology, and the whole of the introduced plasmid is incorporated on the chromosome. After that, if further recombination occurs at the place of the sequence having the homology on the chromosome, the plasmid falls off from the chromosome again. However, during this process, the gene with introduced mutation is occasionally fixed preferentially on the chromosome depending on the position at which recombination takes place, and an original normal gene falls off from the chromosome together with the plasmid. Selection of such microbial strains makes it possible to obtain a microbial strain in which the normal gene on the chromosome is substituted with the modified or destroyed gene obtained by the introduction of mutation having nucleotide substitution, deletion, insertion, addition, or inversion.

The microorganism belonging to the genus Escherichia to be subjected to the gene substitution is a microorganism having L-lysine productivity. The microorganism belonging to the genus Escherichia having L-lysine productivity, for example, a microbial strain of Escherichia coli can be obtained by applying a mutation treatment to a strain having no L-lysine productivity to give it resistance to a lysine analog such as S-(2-aminoethyl)-L-cysteine (hereinafter referred to as "AEC"). Methods for the mutation treatment include methods in which cells of Escherichia coli are subjected to a treatment with a chemical agent such as N-methyl-N'-nitro-N-nitrosoguanidine and nitrous acid, or a treatment with irradiation of ultraviolet light, radiation or the like. Such a microbial strain specifically includes Escherichia coli AJ13069 (FERM P-14690). This microbial strain was bred by giving AEC resistance to W3110 strain originating from Escherichia coli K-12. Escherichia coli AJ13069 was deposited in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology (postal code:305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibarakiken, Japan) under an accession number of FERM P-14690 on December 6, 1994, transferred to international deposition based on the Budapest Treaty on September 29, 1995, and given an accession number of FERM BP-5252.

The microbial strain of Escherichia coli having L-lysine productivity can be also bred by introducing and enhancing DNA which carries genetic information relevant to L-lysine biosynthesis by means of the gene recombination technology. The gene to be introduced are genes which code for enzymes on the biosynthetic pathway of L-lysine, such as aspartokinase, dihydrodipicolinate synthetase, dihydrodipicolinate reductase, succinyldiaminopimelate transaminase, and succinyldiaminopimelate deacylase. In the case of a gene of the enzyme which undergoes feedback inhibition by L-lysine such as aspartokinase and dihydrodipicolinate synthetase, it is desirable to use a mutant type gene coding for an enzyme which is desensitized from such inhibition. In order to introduce and enhance the gene, a method is available, in which the gene is ligated with a vector autonomously replicable in cells of Escherichia coli to prepare recombinant DNA with which Escherichia coli is transformed. Alternatively, the gene can be also incorporated into

chromosome of a host in accordance with a method to use transduction, transposon (Berg, D. E. and Berg, C. M., Bio/Technol., 1, 417 (1983)), Mu phage (Japanese Patent Laid-open No. 2-109985), or homologous recombination (Experiments in Molecular Genetics, Cold Spring Harbor Lab. (1972)).

Other methods to obtain the microorganism belonging to the genus Escherichia with destroyed function of the gene include a method to cause genetic mutation by applying a treatment with a chemical agent such as N-methyl-N'-nitro-N-nitrosoguanidine and nitrous acid, or a treatment with irradiation of ultraviolet light, radiation or the like, to cells of the microorganism belonging to the genus Escherichia having the gene.

In Example described below, an Escherichia coli strain with destroyed function of the lysine decarboxylase gene was created by deleting a part of its coding region, and inserting a drug resistance gene instead of it to obtain a lysine decarboxylase gene which was used to substitute a lysine decarboxylase gene on chromosome of Escherichia coli in accordance with the method utilizing homologous recombination described above.

It is possible to restrain expression of any one of the novel lysine decarboxylase gene of the present invention and cadA gene, or restrain expression of both of them, in one microbial strain. Expression of the lysine decarboxylase gene may be restrained in the microorganism belonging to the genus Escherichia having L-lysine productivity, or L-lysine productivity may be given to the microorganism belonging to the genus Escherichia with restrained expression of the lysine decarboxylase gene in accordance with the method described above.

(3) Production of L-lysine by using microorganism belonging to the genus Escherichia with restrained expression of lysine decarboxylase gene

A considerable amount of L-lysine is produced and accumulated in a culture liquid by cultivating the microorganism belonging to the genus Escherichia with restrained expression of the lysine decarboxylase gene obtained as described above. The accumulation amount of L-lysine is increased only by restraining expression of the known cadA gene. However, it is more effective for increasing the accumulation amount of L-lysine to restrain expression of the novel lysine decarboxylase gene of the present invention. The most preferable result for L-lysine production is obtained by using a microbial strain in which expression of both of the cadA gene and the novel gene of the present invention is restrained.

The medium to be used for L-lysine production is an ordinary medium containing a carbon source, a nitrogen source, inorganic ions, and optionally other organic trace nutrient sources. As the carbon source, it is possible to use sugars such as glucose, lactose, galactose, fructose, and starch hydrolysate; alcohols such as glycerol and sorbitol; and organic acids such as fumaric acid, citric acid, and succinic acid. As the nitrogen source, it is possible to use inorganic ammonium salts such as ammonium sulfate, ammonium chloride, and ammonium phosphate; organic nitrogen sources such as soybean hydrolysate; ammonia gas; and aqueous ammonia. As the inorganic ions, potassium phosphate, magnesium sulfate, iron ion, manganese ion and so on are added in small amounts. Other than the above, it is desirable to contain vitamin B_1, yeast extract or the like in appropriate amounts as the organic trace nutrient sources.

Cultivation is preferably carried out under an aerobic condition for about 16-72 hours. The cultivation temperature is controlled at 30 °C to 45 °C, and pH is controlled at 5-7 during cultivation. Inorganic or organic, acidic or alkaline substances, or ammonia gas or the like can be used for pH adjustment.

After completion of the cultivation, collection of L-lysine from a fermented liquor can be appropriately carried out by combining an ordinary ion exchange resin method, a precipitation method, and other known methods.

Brief Description of the Drawings

Fig. 1 shows a structure of a plasmid pUC6F5HH5 containing the novel lysine decarboxylase gene.

Fig. 2 shows a structure of a temperature-sensitive plasmid pTS6F5HH5 containing the novel lysine decarboxylase gene, and construction of a plasmid pTS6F5HH5Cm in which a part of the gene is substituted with a fragment containing a chloramphenicol resistance gene.

Fig. 3 shows comparison of L-lysine-decomposing activities in a strain WC196 harboring a normal lysine decarboxylase gene, and strains WC196C, WC196L, and WC196LC with destroyed lysine decarboxylase genes.

Best Mode for Carrying Out the Invention

The present invention will be more specifically explained below with reference to Examples.

Example 1

(1) Cloning of novel lysine decarboxylase gene

Chromosomal DNA was extracted in accordance with an ordinary method from cells of W3110 strain of Escherichia coli K-12 obtained from National Institute of Genetics (Yata 1111, Mishima-shi, Shizuoka-ken, Japan). On the other

hand, two synthetic DNA primers as shown in SEQ ID NOS:1 and 2 in Sequence Listing were synthesized in accordance with an ordinary method on the basis of the nucleotide sequence of the cadA gene (see SEQ ID NO:5) described in Meng, S. and Bennett, G. N., J. Bacteriol., 174, 2659 (1992). They had sequences homologous to a 5'-terminal upstream portion and a 3'-terminal portion of the cadA gene respectively. The chromosomal DNA and the DNA primers were used to perform a PCR method in accordance with the method of Erlich et al. (PCR Technology, Stockton press (1989)). Thus a DNA fragment of 2.1 kbp containing almost all parts of the cadA gene was obtained. This fragment was labeled with Random Primer Labeling Kit (produced by Takara Shuzo) and [α-^{32}P]dCTP (produced by Amersham Japan) to prepare a probe for hybridization.

Next, hybridization was performed in accordance with an ordinary method (Molecular Cloning (2nd edition), Cold Spring Harbor Laboratory press (1989)) by using the prepared probe and Escherichia coli/Gene Mapping Membrane (produced by Takara Shuzo). A library of Kohara et al. (lambda phage library of Escherichia coli chromosomal DNA: see Kohara, Y. et al. Cell, 50, 495-508 (1987)) had been adsorbed to Escherichia coli/Gene Mapping Membrane. Lambda phage clones having sequences similar to the cadA gene were screened by weakening the condition for washing the probe (2 x SSC, 55 °C, 30 minutes), when the hybridization was performed. As a result, we succeeded in finding weak signals from three clones of E2B8, 6F5H, and 10F9, in addition to strong signals from clones containing the cadA gene region (21H11, 5G7). Insertion sequences of the three lambda phage clones of E2B8, 6F5H, and 10F9 continue on chromosome of Escherichia coli while overlapping with each other. Thus lambda phage DNA of 6F5H belonging to the library of Kohara et al. (Kohara, Y. et al. Cell, 50, 495-508 (1987)) was separated in accordance with an ordinary method, which was digested with various restriction enzymes to perform Southern blot hybridization by using the probe described above in accordance with a method similar to one described above. As a result, it was revealed that a sequence similar to the cadA gene was present in a DNA fragment of about 5 kbp obtained by digestion with HindIII.

Thus, the fragment of about 5 kbp obtained by digesting the lambda phage DNA of 6F5H with HindIII was ligated with a HindIII digest of a plasmid pUC19 (produced by Takara Shuzo) by using T4 DNA ligase. This reaction mixture was used to transform Escherichia coli JM109 (produced by Takara Shuzo) to obtain ampicillin-resistant strains grown on a complete plate medium (containing 10 g of polypeptone, 5 g of yeast extract, and 5 g of sodium chloride in 1 L of water) added with 50 mg/mL ampicillin. A microbial strain was obtained therefrom, which harbored a plasmid with insertion of the fragment of about 5 kbp obtained by digesting the lambda phage DNA of 6F5H with HindIII. A plasmid was extracted from cells thereof, and a plasmid pUC6F5HH5 was obtained. Fig. 1 shows a structure of the plasmid pUC6F5HH5.

Escherichia coli JM109/pUC6F5HH5 harboring this plasmid was designated as AJ13068, deposited in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology under an accession number of FERM P-14689 on December 6, 1994, transferred to international deposition based on the Budapest Treaty on September 29, 1995, and given an accession number of FERM BP-5251.

(2) Determination of nucleotide sequence of novel lysine decarboxylase gene

A nucleotide sequence of a region between restriction enzyme sites of ClaI and HindIII of obtained pUC6F5HH5 was determined in accordance with a method described in Molecular Cloning (2nd edition), Cold Spring Harbor Laboratory press (1989). As a result, it was revealed that the nucleotide sequence shown in SEQ ID NO:3 in Sequence Listing was encoded. This DNA sequence contains an open reading frame which codes for the amino acid sequence shown in SEQ ID NO:4 in Sequence Listing.

(3) Preparation of Escherichia coli having L-lysine productivity

Escherichia coli W3110 was cultivated at 37 °C for 4 hours in a complete medium (containing 10 g of polypeptone, 5 g of yeast extract, and 5 g of sodium chloride in 1 L of water) to obtain microbial cells which were subjected to a mutation treatment at 37 °C for 30 minutes in a solution of N-methyl-N'-nitro-N-nitrosoguanidine at a concentration of 200 μg/ml, washed, and then applied to a minimum plate medium (containing 7 g of disodium hydrogenphosphate, 3 g of potassium dihydrogenphosphate, 1 g of ammonium chloride, 0.5 g of sodium chloride, 5 g of glucose, 0.25 g of magnesium sulfate hepta-hydrate, and 15 g of agar in 1 L of water) added with 5 g/L of AEC. AEC-resistant strains were obtained by separating colonies appeared after cultivation at 37 °C for 48 hours. WC196 strain as one strain among them had L-lysine productivity. WC196 strain was designated as AJ13069, deposited in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology under an accession number of FERM P-14690 on December 6, 1994, transferred to international deposition based on the Budapest Treaty on September 29, 1995, and given an accession number of FERM BP-5252.

(4) Creation of WC196 strain with destroyed function of novel lysine decarboxylase gene

The fragment of about 5 kbp obtained by digesting the lambda phage DNA of 6F5H with HindIII described above

was ligated with a HindIII digest of a temperature-sensitive plasmid pMAN031 (Yasueda, H. et al., Appl. Microbiol. Biotechnol., 36, 211 (1991)) by using T4 DNA ligase. This reaction mixture was used to transform Escherichia coli JM109, followed by cultivation at 37 °C for 24 hours on a complete plate medium added with 50 mg/L of ampicillin to grow ampicillin-resistant strains. A microbial strain was obtained therefrom, which harbored a plasmid with insertion of the fragment of about 5 kbp obtained by digesting the lambda phage DNA of 6F5H with HindIII. A plasmid was extracted from cells of this strain, and a plasmid pTS6F5HH5 was obtained. The plasmid pTS6F5HH5 was digested with EcoRV to remove a DNA fragment of about 1 kbp. Next, T4 ligase was used to insert a fragment having a chloramphenicol resistance gene of about 1 kbp obtained by digesting pHSG399 (produced by Takara Shuzo) with AccI. Thus a plasmid pTS6F5HH5Cm was constructed. As a result of the operation described above, we succeeded in construction of the plasmid having a DNA fragment with destroyed function of the novel lysine decarboxylase gene. Fig. 2 shows a structure of the plasmid pTS6F5HH5, and the plasmid pTS6F5HH5Cm.

Next, a strain was created, in which the novel lysine decarboxylase gene on chromosome of WC196 strain was substituted with the DNA fragment with destroyed function of the novel lysine decarboxylase gene, in accordance with a general homologous recombination technique (Matsuyama, S. and Mizushima, S., J. Bacteriol., 162, 1196 (1985)) by utilizing the property of temperature sensitivity of the plasmid pTS6F5HH5Cm. Namely, WC196 strain was transformed with the plasmid pTS6F5HH5Cm to firstly obtain a strain which was resistant to ampicillin and resistant to chloramphenicol at 30 °C. Next, this strain was used to obtain a strain which was resistant to ampicillin and resistant to chloramphenicol at 42 °C. Further, this strain was used to obtain a strain which was sensitive to ampicillin and resistant to chloramphenicol at 30 °C. Thus the strain as described above was created, in which the novel lysine decarboxylase gene on chromosome of WC196 strain was substituted with the DNA fragment with destroyed function of the novel lysine decarboxylase gene. This strain was designated as WC196L strain.

(5) Creation of WC196 strain and WC196L strain with deficiency of cadA gene

Escherichia coli, in which cadA as the known lysine decarboxylase gene is destroyed, is already known, including, for example, GNB10181 strain originating from Escherichia coli K-12 (see Auger, E. A. et al., Mol. Microbiol., 3, 609 (1989); this microbial strain is available from, for example, E. coli Genetic Stock Center (Connecticut, USA)). It has been revealed that the region of the cadA gene is deficient in this microbial strain. Thus the character of cadA gene deficiency of GNB10181 strain was transduced into WC196 strain in accordance with a general method by using P1 phage (A Short Course in Bacterial Genetics, Cold Spring Harbor Laboratory Press (1992)) to create WC196C strain. Deficiency of the cadA gene of WC196 strain was confirmed by Southern blot hybridization. In addition, WC196LC strain with deficiency of the cadA gene was created from WC196L strain in accordance with a method similar to one described above.

Example 2

(1) Confirmation of L-lysine-decomposing activities of WC196, WC196C, WC196L, and WC196LC strains

The four created strains described above were cultivated at 37 °C for 17 hours by using a medium for L-lysine production (containing 40 g of glucose, 16 g of ammonium sulfate, 1 g of potassium dihydrogenphosphate, 2 g of yeast extract, 10 mg of manganese sulfate tetra-to penta-hydrate, and 10 mg of iron sulfate heptahydrate in 1 L of water; pH was adjusted to 7.0 with potassium hydroxide, and then 30 g of separately sterilized calcium carbonate was added). Recovered microbial cells were washed twice with a physiological saline solution, suspended in a medium for assaying L-lysine decomposition (containing 17 g of disodium hydrogenphosphate dodeca-hydrate, 3 g of potassium dihydrogenphosphate, 0.5 g of sodium chloride, and 10 g of L-lysine hydrochloride in 1 L of water), and cultivated at 37 °C for 31 hours.

Fig. 3 shows changes in remaining L-lysine amounts in culture liquids in accordance with the passage of time. The amount of L-lysine was quantitatively determined by using Biotech Analyzer AS-210 (produced by Asahi Chemical Industry). Significant decomposition of L-lysine was observed in WC196 strain. However, the decomposing activity was decreased a little in WC196C strain with deficiency of the cadA gene as the known lysine decarboxylase gene. Decomposition of L-lysine was not observed in WC196L and WC196LC strains with destroyed function of the novel lysine decarboxylase gene. Remaining L-lysine in the culture liquid decreased during a period up to about 3 hours of cultivation in any of the microbial strains. However, this phenomenon was caused by incorporation of L-lysine into microbial cells, and not caused by decomposition.

(2) Production of L-lysine by WC196, WC196C, WC196L, and WC196LC strains

The four strains described above were cultivated at 37 °C for 20 hours in the medium for L-lysine production described above. The amounts of L-lysine and cadaverine produced and accumulated in culture liquids were measured. The amount of L-lysine was quantitatively determined by using Biotech Analyzer AS-210 as described above. The

amount of cadaverine was quantitatively determined by using high performance liquid chromatography.

Results are shown in Table 1. The accumulation of L-lysine was increased, and the accumulation of cadaverine as a decomposition product of L-lysine was decreased in WC196C strain with destruction of the cadA gene as compared with WC196 strain, and in WC196L strain with destroyed function of the novel lysine decarboxylase gene as compared with WC196 and WC196C strains. The accumulation of L-lysine was further increased, and the accumulation of cadaverine as a decomposition product of L-lysine was not detected in WC196LC strain with destroyed function of the both lysine decarboxylase genes.

Table 1

Microbial strain	L-lysine accumulation (g/L)	Cadaverine accumulation (g/L)
WC196	1.4	0.6
WC196C	1.9	0.4
WC196L	2.3	0.1
WC196LC	3.3	not detected

Example 3

Escherichia coli WC196LC with disappeared L-lysine-decomposing activity was transformed with pUC6F5HH5 containing the novel lysine decarboxylase gene to obtain an ampicillin-resistant strain. WC196LC strain and WC196LC/pUC6F5HH5 strain were cultivated at 37 °C for 16 hours in a medium for L-lysine production added with 5 g/L of L-lysine, and the amount of produced cadaverine was measured.

Results are shown in Table 2. WC196LC strain failed to convert L-lysine into cadaverine, while WC196LC/pUC6F5HH5 strain had an ability to convert L-lysine into cadaverine.

Table 2

Microbial strain	Production amount of cadaverine (g/L)
WC196LC	not detected
WC196LC/pUC6F5HH5	0.93

Industrial Applicability

The novel lysine decarboxylase gene of the present invention participates in decomposition of L-lysine in Escherichia coli. L-lysine can be produced inexpensively and efficiently by cultivating the bacterium belonging to the genus Escherichia having L-lysine productivity with restrained expression of the gene described above and/or the cadA gene.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) APPLICANT: AJINOMOTO Co., Inc.
(ii) TITLE OF INVENTION: NOVEL LYSINE DECARBOXYLASE GENE AND METHOD OF PRODUCING L-LYSINE
(iii) NUMBER OF SEQUENCES: 6
(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ajinomoto Co., Ltd.
(B) STREET: 15-1, Kyobashi 1-chome, Chuo-ku
(C) CITY: Tokyo 104
(D) STATE:
(E) COUNTRY: Japan
(F) ZIP:
(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: FastSEQ Version 1.5
(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 95 938 648.3
(B) FILING DATE: 05.12.95
(C) CLASSIFICATION:
(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 6-306386
(B) FILING DATE: 09.12.94
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Strehl Schübel-Hopf Groening & Partner
(B) REGISTRATION NUMBER: 94
(ix) TELECOMMUNICATION INFORMATION: EPN-43688
(A) TELEPHONE: [49](89)223911
(B) TELEFAX: [49](89)22 39 15

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
TGGATAACCA CACCGCGTCT 20

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: YES
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
GGAAGGATCA TATTGGCGTT 20

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3269 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli
(B) STRAIN: W3110
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1005..3143
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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ATCGATTCTC TGACTGCGGT TAGCCGTCAG GATGAGAAAC TGGATATTAA CATCGATGAA     60
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GGTGCATGGC AGATTGCGCA ACTGGCACGC CATCCACAGC GTCCTTATAC CCTGGATTAC    180
GTTCGCCTGG CATTTGATGA ATTTGACGAA CTGGCTGGCG ACCGCGCGTA TGCAGACGAT    240
AAAGCTATCG TCGGTGGTAT CGCCCGTCTC GATGGTCGTC CGGTGATGAT CATTGGTCAT    300
CAAAAAGGTC GTGAAACCAA AGAAAAAATT CGCCGTAACT TTGGTATGCC AGCGCCAGAA    360
GGTTACCGCA AAGCACTGCG TCTGATGCAA ATGGCTGAAC GCTTTAAGAT GCCTATCATC    420
ACCTTTATCG ACACCCCGGG GGCTTATCCT GGCGTGGGCG CAGAAGAGCG TGGTCAGTCT    480
GAAGCCATTG CACGCAACCT GCGTGAAATG TCTCGCCTCG GCGTACCGGT AGTTTGTACG    540
GTTATCGGTG AAGGTGGTTC TGGCGGTGCG CTGGCGATTG GCGTGGGCGA TAAAGTGAAT    600
ATGCTGCAAT ACAGCACCTA TTCCGTTATC TCGCCGGAAG GTTGTGCGTC CATTCTGTGG    660
AAGAGCGCCG ACAAAGCGCC GCTGGCGGCT GAAGCGATGG GTATCATTGC TCCGCGTCTG    720
AAAGAACTGA AACTGATCGA CTCCATCATC CCGGAACCAC TGGGTGGTGC TCACCGTAAC    780
CCGGAAGCGA TGGCGGCATC GTTGAAAGCG CAACTGCTGG CGGATCTGGC CGATCTCGAC    840
GTGTTAAGCA CTGAAGATTT AAAAAATCGT CGTTATCAGC GCCTGATGAG CTACGGTTAC    900
GCGTAATTCG CAAAAGTTCT GAAAAAGGGT CACTTCGGTG GCCCTTTTTT ATCGCCACGG    960
TTTGAGCAGG CTATGATTAA GGAAGGATTT TCCAGGAGGA ACAC ATG AAC ATC ATT   1016
                                                 Met Asn Ile Ile
                                                   1
GCC ATT ATG GGA CCG CAT GGC GTC TTT TAT AAA GAT GAG CCC ATC AAA     1064
Ala Ile Met Gly Pro His Gly Val Phe Tyr Lys Asp Glu Pro Ile Lys
  5                  10                  15                  20
GAA CTG GAG TCG GCG CTG GTG GCG CAA GGC TTT CAG ATT ATC TGG CCA     1112
Glu Leu Glu Ser Ala Leu Val Ala Gln Gly Phe Gln Ile Ile Trp Pro
                 25                  30                  35
CAA AAC AGC GTT GAT TTG CTG AAA TTT ATC GAG CAT AAC CCT CGA ATT     1160
Gln Asn Ser Val Asp Leu Leu Lys Phe Ile Glu His Asn Pro Arg Ile
             40                  45                  50
TGC GGC GTG ATT TTT GAC TGG GAT GAG TAC AGT CTC GAT TTA TGT AGC     1208
Cys Gly Val Ile Phe Asp Trp Asp Glu Tyr Ser Leu Asp Leu Cys Ser
         55                  60                  65
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GAT ATC AAT CAG CTT AAT GAA TAT CTC CCG CTT TAT GCC TTC ATC AAC     1256
Asp Ile Asn Gln Leu Asn Glu Tyr Leu Pro Leu Tyr Ala Phe Ile Asn
     70                  75                  80
ACC CAC TCG ACG ATG GAT GTC AGC GTG CAG GAT ATG CGG ATG GCG CTC     1304
Thr His Ser Thr Met Asp Val Ser Val Gln Asp Met Arg Met Ala Leu
 85                  90                  95                 100
TGG TTT TTT GAA TAT GCG CTG GGG CAG GCG GAA GAT ATC GCC ATT CGT     1352
Trp Phe Phe Glu Tyr Ala Leu Gly Gln Ala Glu Asp Ile Ala Ile Arg
                105                 110                 115
ATG CGT CAG TAC ACC GAC GAA TAT CTT GAT AAC ATT ACA CCG CCG TTC     1400
Met Arg Gln Tyr Thr Asp Glu Tyr Leu Asp Asn Ile Thr Pro Pro Phe
            120                 125                 130
ACG AAA GCC TTG TTT ACC TAC GTC AAA GAG CGG AAG TAC ACC TTT TGT     1448
Thr Lys Ala Leu Phe Thr Tyr Val Lys Glu Arg Lys Tyr Thr Phe Cys
        135                 140                 145
ACG CCG GGG CAT ATG GGC GGC ACC GCA TAT CAA AAA AGC CCG GTT GGC     1496
Thr Pro Gly His Met Gly Gly Thr Ala Tyr Gln Lys Ser Pro Val Gly
    150                 155                 160
TGT CTG TTT TAT GAT TTT TTC GGC GGG AAT ACT CTT AAG GCT GAT GTC     1544
Cys Leu Phe Tyr Asp Phe Phe Gly Gly Asn Thr Leu Lys Ala Asp Val
165                 170                 175                 180
TCT ATT TCG GTC ACC GAG CTT GGT TCG TTG CTC GAC CAC ACC GGG CCA     1592
Ser Ile Ser Val Thr Glu Leu Gly Ser Leu Leu Asp His Thr Gly Pro
                185                 190                 195
CAC CTG GAA GCG GAA GAG TAC ATC GCG CGG ACT TTT GGC GCG GAA CAG     1640
His Leu Glu Ala Glu Glu Tyr Ile Ala Arg Thr Phe Gly Ala Glu Gln
            200                 205                 210
AGT TAT ATC GTT ACC AAC GGA ACA TCG ACG TCG AAC AAA ATT GTG GGT     1688
Ser Tyr Ile Val Thr Asn Gly Thr Ser Thr Ser Asn Lys Ile Val Gly
        215                 220                 225
ATG TAC GCC GCG CCA TCC GGC AGT ACG CTG TTG ATC GAC CGC AAT TGT     1736
Met Tyr Ala Ala Pro Ser Gly Ser Thr Leu Leu Ile Asp Arg Asn Cys
    230                 235                 240
CAT AAA TCG CTG GCG CAT CTG TTG ATG ATG AAC GAT GTA GTG CCA GTC     1784
His Lys Ser Leu Ala His Leu Leu Met Met Asn Asp Val Val Pro Val
245                 250                 255                 260
TGG CTG AAA CCG ACG CGT AAT GCG TTG GGG ATT CTT GGT GGG ATC CCG     1832
Trp Leu Lys Pro Thr Arg Asn Ala Leu Gly Ile Leu Gly Gly Ile Pro
                265                 270                 275
CGC CGT GAA TTT ACT CGC GAC AGC ATC GAA GAG AAA GTC GCT GCT ACC     1880
Arg Arg Glu Phe Thr Arg Asp Ser Ile Glu Glu Lys Val Ala Ala Thr
            280                 285                 290
ACG CAA GCA CAA TGG CCG GTT CAT GCG GTG ATC ACC AAC TCC ACC TAT     1928
Thr Gln Ala Gln Trp Pro Val His Ala Val Ile Thr Asn Ser Thr Tyr
        295                 300                 305
GAT GGC TTG CTC TAC AAC ACC GAC TGG ATC AAA CAG ACG CTG GAT GTC     1976
Asp Gly Leu Leu Tyr Asn Thr Asp Trp Ile Lys Gln Thr Leu Asp Val
    310                 315                 320
CCG TCG ATT CAC TTC GAT TCT GCC TGG GTG CCG TAC ACC CAT TTT CAT     2024
Pro Ser Ile His Phe Asp Ser Ala Trp Val Pro Tyr Thr His Phe His
325                 330                 335                 340
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CCG ATC TAC CAG GGT AAA AGT GGT ATG AGC GGC GAG CGT GTT GCG GGA    2072
Pro Ile Tyr Gln Gly Lys Ser Gly Met Ser Gly Glu Arg Val Ala Gly
                345                 350                 355
AAA GTG ATC TTC GAA ACG CAA TCG ACC CAC AAA ATG CTG GCG GCG TTA    2120
Lys Val Ile Phe Glu Thr Gln Ser Thr His Lys Met Leu Ala Ala Leu
            360                 365                 370
TCG CAG GCT TCG CTG ATC CAC ATT AAA GGC GAG TAT GAC GAA GAG GCC    2168
Ser Gln Ala Ser Leu Ile His Ile Lys Gly Glu Tyr Asp Glu Glu Ala
        375                 380                 385
TTT AAC GAA GCC TTT ATG ATG CAT ACC ACC ACC TCG CCC AGT TAT CCC    2216
Phe Asn Glu Ala Phe Met Met His Thr Thr Thr Ser Pro Ser Tyr Pro
    390                 395                 400
ATT GTT GCT TCG GTT GAG ACG GCG GCG GCG ATG CTG CGT GGT AAT CCG    2264
Ile Val Ala Ser Val Glu Thr Ala Ala Ala Met Leu Arg Gly Asn Pro
405                 410                 415                 420
GGC AAA CGG CTG ATT AAC CGT TCA GTA GAA CGA GCT CTG CAT TTT CGC    2312
Gly Lys Arg Leu Ile Asn Arg Ser Val Glu Arg Ala Leu His Phe Arg
                425                 430                 435
AAA GAG GTC CAG CGG CTG CGG GAA GAG TCT GAC GGT TGG TTT TTC GAT    2360
Lys Glu Val Gln Arg Leu Arg Glu Glu Ser Asp Gly Trp Phe Phe Asp
            440                 445                 450
ATC TGG CAA CCG CCG CAG GTG GAT GAA GCC GAA TGC TGG CCC GTT GCG    2408
Ile Trp Gln Pro Pro Gln Val Asp Glu Ala Glu Cys Trp Pro Val Ala
        455                 460                 465
CCT GGC GAA CAG TGG CAC GGC TTT AAC GAT GCG GAT GCC GAT CAT ATG    2456
Pro Gly Glu Gln Trp His Gly Phe Asn Asp Ala Asp Ala Asp His Met
    470                 475                 480
TTT CTC GAT CCG GTT AAA GTC ACT ATT TTG ACA CCG GGG ATG GAC GAG    2504
Phe Leu Asp Pro Val Lys Val Thr Ile Leu Thr Pro Gly Met Asp Glu
485                 490                 495                 500
CAG GGC AAT ATG AGC GAG GAG GGG ATC CCG GCG GCG CTG GTA GCA AAA    2552
Gln Gly Asn Met Ser Glu Glu Gly Ile Pro Ala Ala Leu Val Ala Lys
                505                 510                 515
TTC CTC GAC GAA CGT GGG ATC GTA GTA GAG AAA ACC GGC CCT TAT AAC    2600
Phe Leu Asp Glu Arg Gly Ile Val Val Glu Lys Thr Gly Pro Tyr Asn
            520                 525                 530
CTG CTG TTT CTC TTT AGT ATT GGC ATC GAT AAA ACC AAA GCA ATG GGA    2648
Leu Leu Phe Leu Phe Ser Ile Gly Ile Asp Lys Thr Lys Ala Met Gly
        535                 540                 545
TTA TTG CGT GGG TTG ACG GAA TTC AAA CGC TCT TAC GAT CTC AAC CTG    2696
Leu Leu Arg Gly Leu Thr Glu Phe Lys Arg Ser Tyr Asp Leu Asn Leu
    550                 555                 560
CGG ATC AAA AAT ATG CTA CCC GAT CTC TAT GCA GAA GAT CCC GAT TTC    2744
Arg Ile Lys Asn Met Leu Pro Asp Leu Tyr Ala Glu Asp Pro Asp Phe
565                 570                 575                 580
TAC CGC AAT ATG CGT ATT CAG GAT CTG GCA CAA GGG ATC CAT AAG CTG    2792
Tyr Arg Asn Met Arg Ile Gln Asp Leu Ala Gln Gly Ile His Lys Leu
                585                 590                 595
ATT CGT AAA CAC GAT CTT CCC GGT TTG ATG TTG CGG GCA TTC GAT ACT    2840
Ile Arg Lys His Asp Leu Pro Gly Leu Met Leu Arg Ala Phe Asp Thr
            600                 605                 610
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TTG CCG GAG ATG ATC ATG ACG CCA CAT CAG GCA TGG CAA CGA CAA ATT     2888
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        615                 620                 625
AAA GGC GAA GTA GAA ACC ATT GCG CTG GAA CAA CTG GTC GGT AGA GTA     2936
Lys Gly Glu Val Glu Thr Ile Ala Leu Glu Gln Leu Val Gly Arg Val
    630                 635                 640
TCG GCA AAT ATG ATC CTG CCT TAT CCA CCG GGC GTA CCG CTG TTG ATG     2984
Ser Ala Asn Met Ile Leu Pro Tyr Pro Pro Gly Val Pro Leu Leu Met
645                 650                 655                 660
CCT GGA GAA ATG CTG ACC AAA GAG AGC CGC ACA GTA CTC GAT TTT CTA     3032
Pro Gly Glu Met Leu Thr Lys Glu Ser Arg Thr Val Leu Asp Phe Leu
                665                 670                 675
CTG ATG CTT TGT TCC GTC GGG CAA CAT TAC CCC GGT TTT GAA ACG GAT     3080
Leu Met Leu Cys Ser Val Gly Gln His Tyr Pro Gly Phe Glu Thr Asp
            680                 685                 690
ATT CAC GGC GCG AAA CAG GAC GAA GAC GGC GTT TAC CGC GTA CGA GTC     3128
Ile His Gly Ala Lys Gln Asp Glu Asp Gly Val Tyr Arg Val Arg Val
        695                 700                 705
CTA AAA ATG GCG GGA TAACTTGCCA GAGCGGCTTC CGGGCGAGTA ACGTTCTGTT     3183
Leu Lys Met Ala Gly
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 713 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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            20                  25                  30
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        35                  40                  45
Asn Pro Arg Ile Cys Gly Val Ile Phe Asp Trp Asp Glu Tyr Ser Leu
    50                  55                  60
Asp Leu Cys Ser Asp Ile Asn Gln Leu Asn Glu Tyr Leu Pro Leu Tyr
65                  70                  75                  80
Ala Phe Ile Asn Thr His Ser Thr Met Asp Val Ser Val Gln Asp Met
                85                  90                  95
Arg Met Ala Leu Trp Phe Phe Glu Tyr Ala Leu Gly Gln Ala Glu Asp
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Ile Ala Ile Arg Met Arg Gln Tyr Thr Asp Glu Tyr Leu Asp Asn Ile
        115                 120                 125
Thr Pro Pro Phe Thr Lys Ala Leu Phe Thr Tyr Val Lys Glu Arg Lys
    130                 135                 140
Tyr Thr Phe Cys Thr Pro Gly His Met Gly Gly Thr Ala Tyr Gln Lys
145                 150                 155                 160
Ser Pro Val Gly Cys Leu Phe Tyr Asp Phe Phe Gly Gly Asn Thr Leu
                165                 170                 175
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        195                 200                 205
Gly Ala Glu Gln Ser Tyr Ile Val Thr Asn Gly Thr Ser Thr Ser Asn
    210                 215                 220
Lys Ile Val Gly Met Tyr Ala Ala Pro Ser Gly Ser Thr Leu Leu Ile
225                 230                 235                 240
Asp Arg Asn Cys His Lys Ser Leu Ala His Leu Leu Met Met Asn Asp
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Val Val Pro Val Trp Leu Lys Pro Thr Arg Asn Ala Leu Gly Ile Leu
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Gly Gly Ile Pro Arg Arg Glu Phe Thr Arg Asp Ser Ile Glu Glu Lys
        275                 280                 285
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    290                 295                 300
Asn Ser Thr Tyr Asp Gly Leu Leu Tyr Asn Thr Asp Trp Ile Lys Gln
305                 310                 315                 320
Thr Leu Asp Val Pro Ser Ile His Phe Asp Ser Ala Trp Val Pro Tyr
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Thr His Phe His Pro Ile Tyr Gln Gly Lys Ser Gly Met Ser Gly Glu
            340                 345                 350
Arg Val Ala Gly Lys Val Ile Phe Glu Thr Gln Ser Thr His Lys Met
        355                 360                 365
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    370                 375                 380
Asp Glu Glu Ala Phe Asn Glu Ala Phe Met Met His Thr Thr Thr Ser
385                 390                 395                 400
Pro Ser Tyr Pro Ile Val Ala Ser Val Glu Thr Ala Ala Ala Met Leu
                405                 410                 415
Arg Gly Asn Pro Gly Lys Arg Leu Ile Asn Arg Ser Val Glu Arg Ala
            420                 425                 430
Leu His Phe Arg Lys Glu Val Gln Arg Leu Arg Glu Glu Ser Asp Gly
        435                 440                 445
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    450                 455                 460
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465                 470                 475                 480
Ala Asp His Met Phe Leu Asp Pro Val Lys Val Thr Ile Leu Thr Pro
                485                 490                 495
Gly Met Asp Glu Gln Gly Asn Met Ser Glu Glu Gly Ile Pro Ala Ala
            500                 505                 510
Leu Val Ala Lys Phe Leu Asp Glu Arg Gly Ile Val Val Glu Lys Thr
        515                 520                 525
Gly Pro Tyr Asn Leu Leu Phe Leu Phe Ser Ile Gly Ile Asp Lys Thr
    530                 535                 540
Lys Ala Met Gly Leu Leu Arg Gly Leu Thr Glu Phe Lys Arg Ser Tyr
545                 550                 555                 560
Asp Leu Asn Leu Arg Ile Lys Asn Met Leu Pro Asp Leu Tyr Ala Glu
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Asp Pro Asp Phe Tyr Arg Asn Met Arg Ile Gln Asp Leu Ala Gln Gly
            580                 585                 590
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Ile His Lys Leu Ile Arg Lys His Asp Leu Pro Gly Leu Met Leu Arg
        595                 600                 605
Ala Phe Asp Thr Leu Pro Glu Met Ile Met Thr Pro His Gln Ala Trp
    610                 615                 620
Gln Arg Gln Ile Lys Gly Glu Val Glu Thr Ile Ala Leu Glu Gln Leu
625                 630                 635                 640
Val Gly Arg Val Ser Ala Asn Met Ile Leu Pro Tyr Pro Pro Gly Val
                645                 650                 655
Pro Leu Leu Met Pro Gly Glu Met Leu Thr Lys Glu Ser Arg Thr Val
            660                 665                 670
Leu Asp Phe Leu Leu Met Leu Cys Ser Val Gly Gln His Tyr Pro Gly
        675                 680                 685
Phe Glu Thr Asp Ile His Gly Ala Lys Gln Asp Glu Asp Gly Val Tyr
    690                 695                 700
Arg Val Arg Val Leu Lys Met Ala Gly
705                 710
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(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli
(B) STRAIN: CS520
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2145
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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  1               5                  10                  15
GAA CCC ATC CGT GAA CTT CAT CGC GCG CTT GAA CGT CTG AAC TTC CAG        96
Glu Pro Ile Arg Glu Leu His Arg Ala Leu Glu Arg Leu Asn Phe Gln
             20                  25                  30
ATT GTT TAC CCG AAC GAC CGT GAC GAC TTA TTA AAA CTG ATC GAA AAC       144
Ile Val Tyr Pro Asn Asp Arg Asp Asp Leu Leu Lys Leu Ile Glu Asn
         35                  40                  45
AAT GCG CGT CTG TGC GGC GTT ATT TTT GAC TGG GAT AAA TAT AAT CTC       192
Asn Ala Arg Leu Cys Gly Val Ile Phe Asp Trp Asp Lys Tyr Asn Leu
     50                  55                  60
GAG CTG TGC GAA GAA ATT AGC AAA ATG AAC GAG AAC CTG CCG TTG TAC       240
Glu Leu Cys Glu Glu Ile Ser Lys Met Asn Glu Asn Leu Pro Leu Tyr
 65                  70                  75                  80
GCG TTC GCT AAT ACG TAT TCC ACT CTC GAT GTA AGC CTG AAT GAC CTG       288
Ala Phe Ala Asn Thr Tyr Ser Thr Leu Asp Val Ser Leu Asn Asp Leu
                 85                  90                  95
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CGT TTA CAG ATT AGC TTC TTT GAA TAT GCG CTG GGT GCT GCT GAA GAT       336
Arg Leu Gln Ile Ser Phe Phe Glu Tyr Ala Leu Gly Ala Ala Glu Asp
            100                 105                 110
ATT GCT AAT AAG ATC AAG CAG ACC ACT GAC GAA TAT ATC AAC ACT ATT       384
Ile Ala Asn Lys Ile Lys Gln Thr Thr Asp Glu Tyr Ile Asn Thr Ile
        115                 120                 125
CTG CCT CCG CTG ACT AAA GCA CTG TTT AAA TAT GTT CGT GAA GGT AAA       432
Leu Pro Pro Leu Thr Lys Ala Leu Phe Lys Tyr Val Arg Glu Gly Lys
    130                 135                 140
TAT ACT TTC TGT ACT CCT GGT CAC ATG GGC GGT ACT GCA TTC CAG AAA       480
Tyr Thr Phe Cys Thr Pro Gly His Met Gly Gly Thr Ala Phe Gln Lys
145                 150                 155                 160
AGC CCG GTA GGT AGC CTG TTC TAT GAT TTC TTT GGT CCG AAT ACC ATG       528
Ser Pro Val Gly Ser Leu Phe Tyr Asp Phe Phe Gly Pro Asn Thr Met
                165                 170                 175
AAA TCT GAT ATT TCC ATT TCA GTA TCT GAA CTG GGT TCT CTG CTG GAT       576
Lys Ser Asp Ile Ser Ile Ser Val Ser Glu Leu Gly Ser Leu Leu Asp
            180                 185                 190
CAC AGT GGT CCA CAC AAA GAA GCA GAA CAG TAT ATC GCT CGC GTC TTT       624
His Ser Gly Pro His Lys Glu Ala Glu Gln Tyr Ile Ala Arg Val Phe
        195                 200                 205
AAC GCA GAC CGC AGC TAC ATG GTG ACC AAC GGT ACT TCC ACT GCG AAC       672
Asn Ala Asp Arg Ser Tyr Met Val Thr Asn Gly Thr Ser Thr Ala Asn
    210                 215                 220
AAA ATT GTT GGT ATG TAC TCT GCT CCA GCA GGC AGC ACC ATT CTG ATT       720
Lys Ile Val Gly Met Tyr Ser Ala Pro Ala Gly Ser Thr Ile Leu Ile
225                 230                 235                 240
GAC CGT AAC TGC CAC AAA TCG CTG ACC CAC CTG ATG ATG ATG AGC GAT       768
Asp Arg Asn Cys His Lys Ser Leu Thr His Leu Met Met Met Ser Asp
                245                 250                 255
GTT ACG CCA ATC TAT TTC CGC CCG ACC CGT AAC GCT TAC GGT ATT CTT       816
Val Thr Pro Ile Tyr Phe Arg Pro Thr Arg Asn Ala Tyr Gly Ile Leu
            260                 265                 270
GGT GGT ATC CCA CAG AGT GAA TTC CAG CAC GCT ACC ATT GCT AAG CGC       864
Gly Gly Ile Pro Gln Ser Glu Phe Gln His Ala Thr Ile Ala Lys Arg
        275                 280                 285
GTG AAA GAA ACA CCA AAC GCA ACC TGG CCG GTA CAT GCT GTA ATT ACC       912
Val Lys Glu Thr Pro Asn Ala Thr Trp Pro Val His Ala Val Ile Thr
    290                 295                 300
AAC TCT ACC TAT GAT GGT CTG CTG TAC AAC ACC GAC TTC ATC AAG AAA       960
Asn Ser Thr Tyr Asp Gly Leu Leu Tyr Asn Thr Asp Phe Ile Lys Lys
305                 310                 315                 320
ACA CTG GAT GTG AAA TCC ATC CAC TTT GAC TCC GCG TGG GTG CCT TAC      1008
Thr Leu Asp Val Lys Ser Ile His Phe Asp Ser Ala Trp Val Pro Tyr
                325                 330                 335
ACC AAC TTC TCA CCG ATT TAC GAA GGT AAA TGC GGT ATG AGC GGT GGC      1056
Thr Asn Phe Ser Pro Ile Tyr Glu Gly Lys Cys Gly Met Ser Gly Gly
            340                 345                 350
CGT GTA GAA GGG AAA GTG ATT TAC GAA ACC CAG TCC ACT CAC AAA CTG      1104
Arg Val Glu Gly Lys Val Ile Tyr Glu Thr Gln Ser Thr His Lys Leu
        355                 360                 365
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CTG GCG GCG TTC TCT CAG GCT TCC ATG ATC CAC GTT AAA GGT GAC GTA      1152
Leu Ala Ala Phe Ser Gln Ala Ser Met Ile His Val Lys Gly Asp Val
    370                 375                 380
AAC GAA GAA ACC TTT AAC GAA GCC TAC ATG ATG CAC ACC ACC ACT TCT      1200
Asn Glu Glu Thr Phe Asn Glu Ala Tyr Met Met His Thr Thr Thr Ser
385                 390                 395                 400
CCG CAC TAC GGT ATC GTG GCG TCC ACT GAA ACC GCT GCG GCG ATG ATG      1248
Pro His Tyr Gly Ile Val Ala Ser Thr Glu Thr Ala Ala Ala Met Met
                405                 410                 415
AAA GGC AAT GCA GGT AAG CGT CTG ATC AAC GGT TCT ATT GAA CGT GCG      1296
Lys Gly Asn Ala Gly Lys Arg Leu Ile Asn Gly Ser Ile Glu Arg Ala
            420                 425                 430
ATC AAA TTC CGT AAA GAG ATC AAA CGT CTG AGA ACG GAA TCT GAT GGC      1344
Ile Lys Phe Arg Lys Glu Ile Lys Arg Leu Arg Thr Glu Ser Asp Gly
        435                 440                 445
TGG TTC TTT GAT GTA TGG CAG CCG GAT CAT ATC GAT ACG ACT GAA TGC      1392
Trp Phe Phe Asp Val Trp Gln Pro Asp His Ile Asp Thr Thr Glu Cys
    450                 455                 460
TGG CCG CTG CGT TCT GAC AGC ACC TGG CAC GGC TTC AAA AAC ATC GAT      1440
Trp Pro Leu Arg Ser Asp Ser Thr Trp His Gly Phe Lys Asn Ile Asp
465                 470                 475                 480
AAC GAG CAC ATG TAT CTT GAC CCG ATC AAA GTC ACC CTG CTG ACT CCG      1488
Asn Glu His Met Tyr Leu Asp Pro Ile Lys Val Thr Leu Leu Thr Pro
                485                 490                 495
GGG ATG GAA AAA GAC GGC ACC ATG AGC GAC TTT GGT ATT CCG GCC AGC      1536
Gly Met Glu Lys Asp Gly Thr Met Ser Asp Phe Gly Ile Pro Ala Ser
            500                 505                 510
ATC GTG GCG AAA TAC CTC GAC GAA CAT GGC ATC GTT GTT GAG AAA ACC      1584
Ile Val Ala Lys Tyr Leu Asp Glu His Gly Ile Val Val Glu Lys Thr
        515                 520                 525
GGT CCG TAT AAC CTG CTG TTC CTG TTC AGC ATC GGT ATC GAT AAG ACC      1632
Gly Pro Tyr Asn Leu Leu Phe Leu Phe Ser Ile Gly Ile Asp Lys Thr
    530                 535                 540
AAA GCA CTG AGC CTG CTG CGT GCT CTG ACT GAC TTT AAA CGT GCG TTC      1680
Lys Ala Leu Ser Leu Leu Arg Ala Leu Thr Asp Phe Lys Arg Ala Phe
545                 550                 555                 560
GAC CTG AAC CTG CGT GTG AAA AAC ATG CTG CCG TCT CTG TAT CGT GAA      1728
Asp Leu Asn Leu Arg Val Lys Asn Met Leu Pro Ser Leu Tyr Arg Glu
                565                 570                 575
GAT CCT GAA TTC TAT GAA AAC ATG CGT ATT CAG GAA CTG GCT CAG AAT      1776
Asp Pro Glu Phe Tyr Glu Asn Met Arg Ile Gln Glu Leu Ala Gln Asn
            580                 585                 590
ATC CAC AAA CTG ATT GTT CAC CAC AAT CTG CCG GAT CTG ATG TAT CGC      1824
Ile His Lys Leu Ile Val His His Asn Leu Pro Asp Leu Met Tyr Arg
        595                 600                 605
GCA TTT GAA GTG CTG CCG ACG ATG GTA ATG ACT CCG TAT GCT GCA TTC      1872
Ala Phe Glu Val Leu Pro Thr Met Val Met Thr Pro Tyr Ala Ala Phe
    610                 615                 620
CAG AAA GAG CTG CAC GGT ATG ACC GAA GAA GTT TAC CTC GAC GAA ATG      1920
Gln Lys Glu Leu His Gly Met Thr Glu Glu Val Tyr Leu Asp Glu Met
625                 630                 635                 640
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GTA GGT CGT ATT AAC GCC AAT ATG ATC CTT CCG TAC CCG CCG GGA GTT      1968
Val Gly Arg Ile Asn Ala Asn Met Ile Leu Pro Tyr Pro Pro Gly Val
                645                 650                 655
CCT CTG GTA ATG CCG GGT GAA ATG ATC ACC GAA GAA AGC CGT CCG GTT      2016
Pro Leu Val Met Pro Gly Glu Met Ile Thr Glu Glu Ser Arg Pro Val
            660                 665                 670
CTG GAG TTC CTG CAG ATG CTG TGT GAA ATC GGC GCT CAC TAT CCG GGC      2064
Leu Glu Phe Leu Gln Met Leu Cys Glu Ile Gly Ala His Tyr Pro Gly
        675                 680                 685
TTT GAA ACC GAT ATT CAC GGT GCA TAC CGT CAG GCT GAT GGC CGC TAT      2112
Phe Glu Thr Asp Ile His Gly Ala Tyr Arg Gln Ala Asp Gly Arg Tyr
    690                 695                 700
ACC GTT AAG GTA TTG AAA GAA GAA AGC AAA AAA                          2145
Thr Val Lys Val Leu Lys Glu Glu Ser Lys Lys
705                 710                 715
```

```
(2) INFORMATION FOR SEQ ID NO:6:
     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 715 amino acids
          (B) TYPE: amino acid
           (D) TOPOLOGY: linear
    (ii) MOLECULE TYPE: protein
    (ix) SEQUENCE DESCRIPTION: SEQ ID NO:6:
Met Asn Val Ile Ala Ile Leu Asn His Met Gly Val Tyr Phe Lys Glu
 1               5                  10                  15
Glu Pro Ile Arg Glu Leu His Arg Ala Leu Glu Arg Leu Asn Phe Gln
            20                  25                  30
Ile Val Tyr Pro Asn Asp Arg Asp Asp Leu Leu Lys Leu Ile Glu Asn
        35                  40                  45
Asn Ala Arg Leu Cys Gly Val Ile Phe Asp Trp Asp Lys Tyr Asn Leu
    50                  55                  60
Glu Leu Cys Glu Glu Ile Ser Lys Met Asn Glu Asn Leu Pro Leu Tyr
 65                 70                  75                  80
Ala Phe Ala Asn Thr Tyr Ser Thr Leu Asp Val Ser Leu Asn Asp Leu
                85                  90                  95
Arg Leu Gln Ile Ser Phe Phe Glu Tyr Ala Leu Gly Ala Ala Glu Asp
            100                 105                 110
Ile Ala Asn Lys Ile Lys Gln Thr Thr Asp Glu Tyr Ile Asn Thr Ile
        115                 120                 125
Leu Pro Pro Leu Thr Lys Ala Leu Phe Lys Tyr Val Arg Glu Gly Lys
    130                 135                 140
Tyr Thr Phe Cys Thr Pro Gly His Met Gly Gly Thr Ala Phe Gln Lys
145                 150                 155                 160
Ser Pro Val Gly Ser Leu Phe Tyr Asp Phe Phe Gly Pro Asn Thr Met
                165                 170                 175
Lys Ser Asp Ile Ser Ile Ser Val Ser Glu Leu Gly Ser Leu Leu Asp
            180                 185                 190
His Ser Gly Pro His Lys Glu Ala Glu Gln Tyr Ile Ala Arg Val Phe
        195                 200                 205
Asn Ala Asp Arg Ser Tyr Met Val Thr Asn Gly Thr Ser Thr Ala Asn
    210                 215                 220
Lys Ile Val Gly Met Tyr Ser Ala Pro Ala Gly Ser Thr Ile Leu Ile
225                 230                 235                 240
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Asp Arg Asn Cys His Lys Ser Leu Thr His Leu Met Met Met Ser Asp
                245                 250                 255
Val Thr Pro Ile Tyr Phe Arg Pro Thr Arg Asn Ala Tyr Gly Ile Leu
            260                 265                 270
Gly Gly Ile Pro Gln Ser Glu Phe Gln His Ala Thr Ile Ala Lys Arg
        275                 280                 285
Val Lys Glu Thr Pro Asn Ala Thr Trp Pro Val His Ala Val Ile Thr
    290                 295                 300
Asn Ser Thr Tyr Asp Gly Leu Leu Tyr Asn Thr Asp Phe Ile Lys Lys
305                 310                 315                 320
Thr Leu Asp Val Lys Ser Ile His Phe Asp Ser Ala Trp Val Pro Tyr
                325                 330                 335
Thr Asn Phe Ser Pro Ile Tyr Glu Gly Lys Cys Gly Met Ser Gly Gly
            340                 345                 350
Arg Val Glu Gly Lys Val Ile Tyr Glu Thr Gln Ser Thr His Lys Leu
        355                 360                 365
Leu Ala Ala Phe Ser Gln Ala Ser Met Ile His Val Lys Gly Asp Val
    370                 375                 380
Asn Glu Glu Thr Phe Asn Glu Ala Tyr Met Met His Thr Thr Thr Ser
385                 390                 395                 400
Pro His Tyr Gly Ile Val Ala Ser Thr Glu Thr Ala Ala Ala Met Met
                405                 410                 415
Lys Gly Asn Ala Gly Lys Arg Leu Ile Asn Gly Ser Ile Glu Arg Ala
            420                 425                 430
Ile Lys Phe Arg Lys Glu Ile Lys Arg Leu Arg Thr Glu Ser Asp Gly
        435                 440                 445
Trp Phe Phe Asp Val Trp Gln Pro Asp His Ile Asp Thr Thr Glu Cys
    450                 455                 460
Trp Pro Leu Arg Ser Asp Ser Thr Trp His Gly Phe Lys Asn Ile Asp
465                 470                 475                 480
Asn Glu His Met Tyr Leu Asp Pro Ile Lys Val Thr Leu Leu Thr Pro
                485                 490                 495
Gly Met Glu Lys Asp Gly Thr Met Ser Asp Phe Gly Ile Pro Ala Ser
            500                 505                 510
Ile Val Ala Lys Tyr Leu Asp Glu His Gly Ile Val Val Glu Lys Thr
        515                 520                 525
Gly Pro Tyr Asn Leu Leu Phe Leu Phe Ser Ile Gly Ile Asp Lys Thr
    530                 535                 540
Lys Ala Leu Ser Leu Leu Arg Ala Leu Thr Asp Phe Lys Arg Ala Phe
545                 550                 555                 560
Asp Leu Asn Leu Arg Val Lys Asn Met Leu Pro Ser Leu Tyr Arg Glu
                565                 570                 575
Asp Pro Glu Phe Tyr Glu Asn Met Arg Ile Gln Glu Leu Ala Gln Asn
            580                 585                 590
Ile His Lys Leu Ile Val His His Asn Leu Pro Asp Leu Met Tyr Arg
        595                 600                 605
Ala Phe Glu Val Leu Pro Thr Met Val Met Thr Pro Tyr Ala Ala Phe
    610                 615                 620
Gln Lys Glu Leu His Gly Met Thr Glu Glu Val Tyr Leu Asp Glu Met
625                 630                 635                 640
Val Gly Arg Ile Asn Ala Asn Met Ile Leu Pro Tyr Pro Pro Gly Val
                645                 650                 655
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Pro Leu Val Met Pro Gly Glu Met Ile Thr Glu Glu Ser Arg Pro Val
            660                 665                 670
Leu Glu Phe Leu Gln Met Leu Cys Glu Ile Gly Ala His Tyr Pro Gly
        675                 680                 685
Phe Glu Thr Asp Ile His Gly Ala Tyr Arg Gln Ala Asp Gly Arg Tyr
    690                 695                 700
Thr Val Lys Val Leu Lys Glu Glu Ser Lys Lys
705                 710                 715
```

Claims

1. A gene which codes for lysine decarboxylase having an amino acid sequence shown in SEQ ID NO:4 in Sequence Listing.

2. The gene according to claim 1, wherein the gene has a nucleotide sequence from 1005th to 3143rd codes shown in SEQ ID NO:3 in Sequence Listing.

3. The gene according to claim 1, wherein said amino acid sequence has substitution, deletion, or insertion of one or a plurality of amino acid residues without any substantial deterioration of lysine decarboxylase activity.

4. A microorganism belonging to the genus Escherichia having L-lysine productivity with decreased or disappeared lysine decarboxylase activity in cells.

5. The microorganism according to claim 4, wherein said microorganism is Escherichia coli.

6. The microorganism according to claim 4, wherein the lysine decarboxylase activity in cells is decreased or disappeared by restraining expression of the gene as defined in any one of claims 1-3 and/or a cadA gene.

7. The microorganism according to claim 6, wherein the expression of the gene is restrained by destroying the gene as defined in any one of claims 1-3 and/or the cadA gene.

8. The microorganism according to claim 6, wherein the gene as defined in any one of claims 1-3 and/or the cadA gene are/is destroyed by substitution, deletion, insertion, addition, or inversion of one or a plurality of nucleotides in the nucleotide sequence or sequences.

9. A method of producing L-lysine comprising the steps of cultivating, in a liquid medium, a microorganism belonging to the genus Escherichia having L-lysine productivity with decreased or disappeared lysine decarboxylase activity in cells to allow L-lysine to be produced and accumulated in a culture liquid, and collecting it.

10. The method according to claim 9, wherein the lysine decarboxylase activity in cells is decreased or disappeared by restraining expression of the gene as defined in any one of claims 1-3 and/or a cadA gene.

FIG. 1

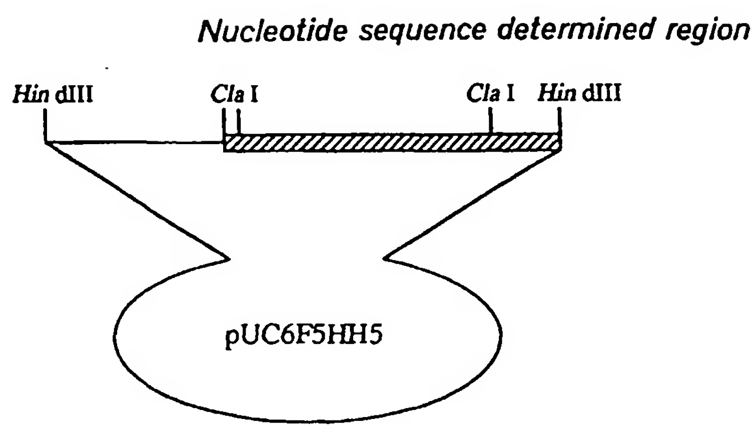

Nucleotide sequence determined region
Hin dIII
Cla I
Cla I
Hin dIII
pUC6F5HH5

FIG.2

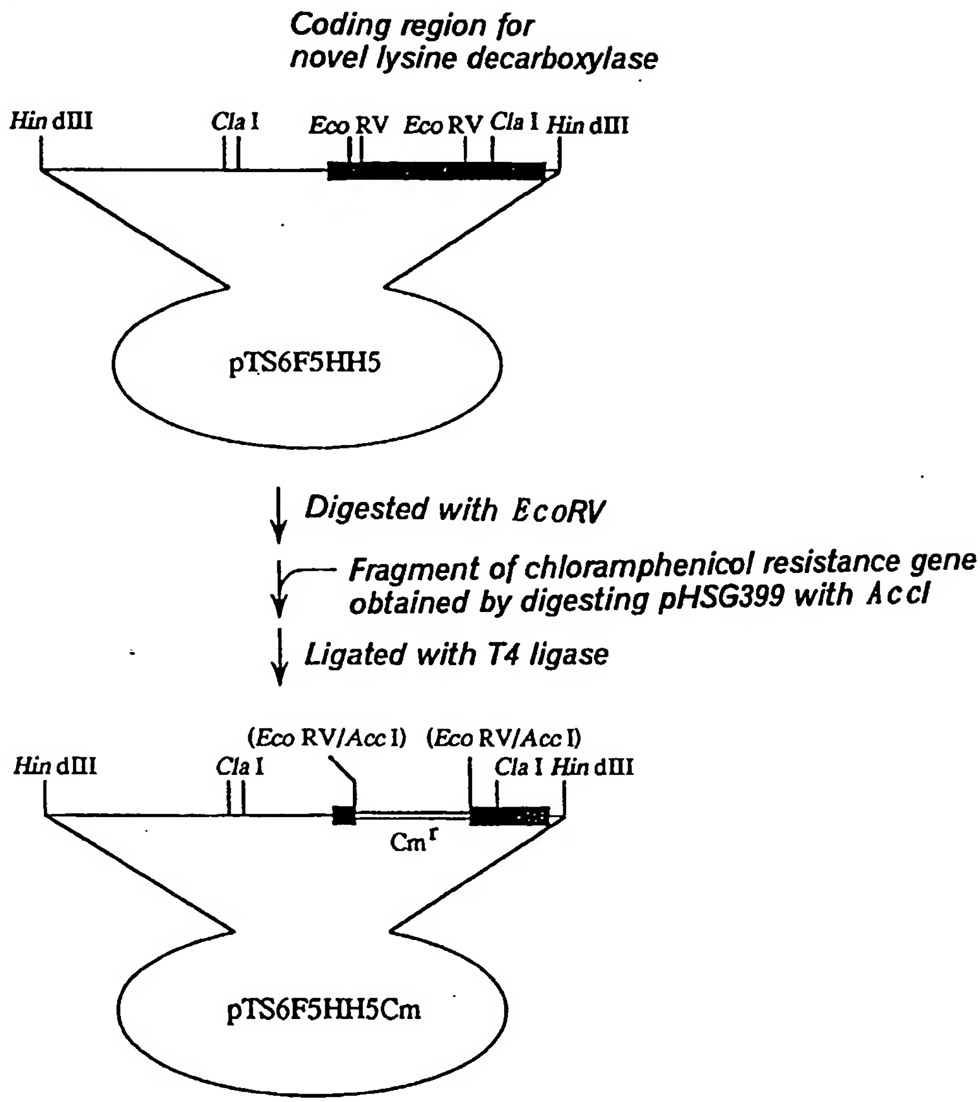
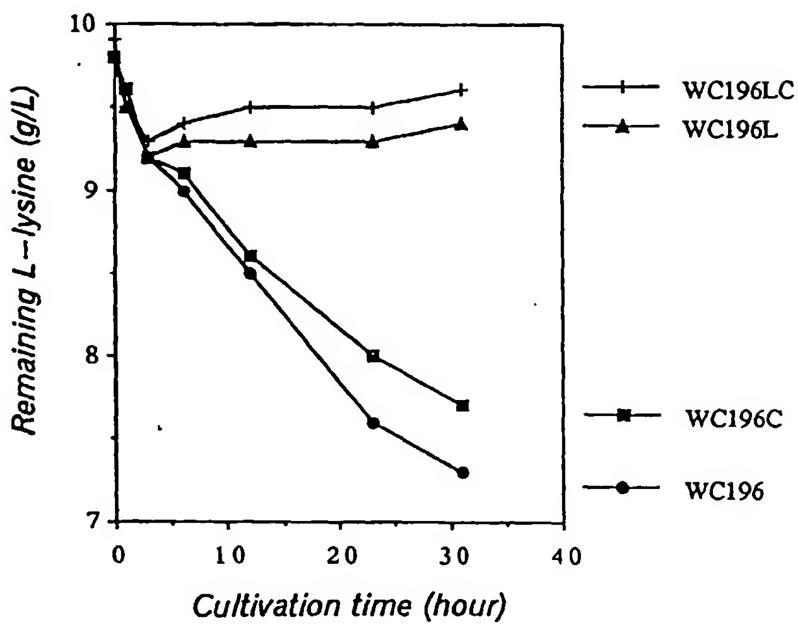

Coding region for
novel lysine decarboxylase
Hin dIII
Cla I
Eco RV
Eco RV
Cla I
Hin dIII
pTS6F5HH5
Digested with EcoRV
Fragment of chloramphenicol resistance gene
obtained by digesting pHSG399 with AccI
Ligated with T4 ligase
(Eco RV/Acc I)
(Eco RV/Acc I)
Hin dIII
Cla I
Cla I
Hin dIII
Cm^r
pTS6F5HH5Cm

FIG.3


10
9
8
7
Remaining L−lysine (g/L)
0
10
20
30
40
Cultivation time (hour)
WC196LC
WC196L
WC196C
WC196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/02481

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl[6] C12N15/00, C12N9/88

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl[6] C12N15/00, C12N9/88

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS PREVIEWS, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STIM K. P. Nucleotide sequence of the ADI gene which encodes the biodegradative acid-induced arginine decarboxylase of Escherichia-coli J. Bacteriol., 1993, Vol. 175, No. 5, p. 1221-1234	1 - 3
A	MENG. S-Y, Nucleotide sequence of the Eseherichia-coli, CAD operon a system for Neutralization of low extracellular PH, J. Bacteriol., 1992, Vol. 174, No. 8, p. 2659-2669	1 - 10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
February 8, 1996 (08. 02. 96)	March 5, 1996 (05. 03. 96)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

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